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(71) Applicant
Orion-Yhtyma Oy

(Incorporated in Finland)

P O Box 65, SF-02101 Espoo, Finland

(72) Inventors
Marjut Ranki
Hans Soderlund

(74) Agent and/or Address for Service
R G C Jenkins & Co,
2 Fetter Lane, London EC4A 1PL

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(54) Quantification of nucleic acid molecules

(57) The invention is related to the quantification of certain nucleic acid molecules, particularly the degree of amplification of genes and/or corresponding messenger RNA molecules using the sandwich or solution hybridization method, and the reagent kit used. The determination is carried out by comparing the number of the test nucleic acid molecules potentially present in several copies in given unit to the number of chosen standard nucleic acid molecules advantageously present in a constant number per same unit.

of the resulting restriction fragments situated relatively close together are cloned to at least two suitable vectors. One of the fragments, the detector probe, is labelled with a suitable detectable label and the other, the capturing probe, is either affixed to a suitable carrier or an substance is affixed to it, which substance enables separation of the resulting hybrid from the hybridization mixture by means of another substance, such as the complementary component of an affinity pair.

The test and standard probe pairs can be assembled into suitable reagent kits wherein the test and standard probe pairs are both DNA or RNA, or the test probe pair is DNA and the standard probe pair RNA or vice versa. The pre- and further treatment of samples prior to hybridization and the hybridization conditions should therefore comply with the probe pairs used in the test.

The method of the present invention is particularly suitable for determining the number of nucleic acid molecules directly from cellular homogenates. The method may of course also be used for the determination of purified or pure nucleic acids. However, before carrying out the method of the invention, the most suitable pretreatment of the nucleic acid sample should be selected.

It is possible to carry out both DNA and RNA determinations using the method of the invention. Deoxyribonucleic acids are denaturated to obtain single strands if necessary. Single-stranded messenger RNA molecules potentially disturbing the hybridization test can be hydrolyzed, for example by alkaline boiling. The sample is not denatured in connection with ribonucleic acid determinations since the double-stranded deoxyribonucleic acid does not interfere with RNA determination. It is of course possible to disrupt the DNA with deoxyribonuclease or alter it either chemically or mechanically so that it cannot participate in the hybridization reaction. Therefore in connection with DNA and RNA determinations a suitable method for further treatment of the sample must be selected or, alternatively, this further treatment may be omitted. The choice of a suitable method for the further treatment is of course dependent on the method used for the preliminary treatment of the nucleic acid sample. Numerous methods of pre- and further treatment of nucleic acid samples have been described in the literature, enabling the most suitable method to be chosen in each case.

Determinations in which both the test and standard nucleic acids are either DNA or RNA can be performed using an undivided sample. Determinations in which the test nucleic acids are DNA and the standard nucleic acids RNA or vice versa must be performed using a divided sample, as different methods for further treatment are necessary. The sample may of course be divided even if the test and standard nucleic acids are of the same nucleic acid type.

The hybridization test itself is performed by bringing the undivided sample solution into contact simultaneously with at least one test probe pair and one standard probe pair. If the sample solution has been divided it is brought separately into contact with at least one test probe pair and one standard probe pair. In such instances, the quantity of test nucleic acid is determined in one reaction vessel and the quantity of the standard nucleic acid in the other.

Regardless of whether the sample is divided or not, hybridization is allowed to take place in the most advantageous conditions and time in each case. Once the reaction(s) has/have taken place, the resulting test and standard hybrids are separated from the hybridization mixture(s) by the carrier and washed, or by an isolation agent such as the complementary member of an affinity pair. The label attached to the test and standard hybrids is measured and the result compared with standard curves. In this way the number of nucleic acid molecules to be studied can be determined per selected unit.

The method of the invention is of practical diagnostic value, particularly in the detection of some types of cancer. In small cell lung carcinoma, the c-myc gene is often amplified and its level of expression considerably higher than in normal tissue. In cases of neuroblastoma the N-myc gene is amplified.

The method of the present invention can also be used for demonstrating the mutagenic or carcinogenic effects of certain agents or the development of drug resistance. It is known that external pressure of selection can result in enhanced expression of a certain gene. In the treatment of cancer, cells develop resistance to a griven drug by amplification of the gene, the expression product of which inactivates the drug. One such case is methotrexate which induces amplification of the gene for dihydrofolate reductase (DHFR). A further example is amplification of the gene for metallothionine under the influence of cadmium.

The expression level of a gene is important from the point of view of the phenotype and function of the cell. This can be investigated by measuring the quantity of messenger RNA which correlates to the quantity of protein coded by it. The transcription product of an oncogene determines the way in which it will ultimately be expressed.

The expression levels of an oncogene vary depending on the cell type, differentiation level and phase of development of the cell. For example, at a certain stage of fetal development, the c-myc oncogene is copied rapidly, whereas at another stage this is very slow. The degree of amplification often correlates with the level of expression of the gene, although the latter may

significantly increase without the former. In such instances the role of an oncogene is best determined by measuring its level of expression rather than the number of copies. In some instances, quantitative determination of the messenger RNA may be simpler and handier than quantification of the gene product itself. As an example the c-myc oncogene, a labile protein readily coagulated by heat, can be mentioned.

The method of the invention can also be used for identifying numerical chromosomal abnormalities such as Down's syndrome. In prenatal diagnostics it is also possible to determine whether the fetus is defective, i.e. homozygous for some recessive gene.

The method of the invention and the nucleic acid reagents used in the method are described in greater detail below.

EXAMPLE 1

a) *Nucleic acid reagents and their preparation*

STANDARD PROBES

Cell standard nucleic acid. The c-Ki-rasl gene is present in all human cells. The probe pairs for sandwich hybridization were prepared by subcloning the HindIII fragment of the c-Ki-rasl gene, measuring 3.8 kb in length, the restriction map of which has been described by Chang et al., PNAS *79*, pp. 4848–52, 1982. The fragment is available e.g. cloned into the pBR322 plasmid (ATCC 41032) and can be obtained e.g. from the ATCC culture collection.

Further treatment of the cell standard nucleic acid. The pBR322 clone described above was treated with BglII and HindIII restriction enzymes and the resulting fragments were isolated from the agarose gel; purified fragments located close together were subcloned into two suitable vectors for preparation of the detector and capturing probes.

Standard detector probe. A BglII-BglII fragment measuring about 1.1 kb in length was subcloned into the BamHI restriction enzyme site of the pBR322 plasmid and labelled by nick-translation with ^{125}I-labelled dCTP.

Standard capturing probe. The BglII-HindIII fragment of about 0.5 kb was inserted into the M13 mp10 and mp11 phage fvectors between the restriction sites of the BamHI and HindIII restriction enzymes and affixed to a nitrocellulose filter (150 ng DNA/dia 1 cm).

TEST PROBES

Test nucleic acid. A probe pair for sandwich hybridization was prepared from a cloned c-myc gene which can be obtained for example, from the ATCC culture collection (ATCC 41010). The restriction map of gene has been described by Watt et al., PNAS *80*, pp. 6307–6311, 1983.

Further treatment of the test nucleic acid. The c-myc gene was treated with HindIII, XbaI and PstI restriction enzymes and the fragments isolated from the agarose gel, purified and subcloned into suitable vectors in order to prepare the detector and capturing probes.

Test detector probe. The single-stranded tails of the HindIII-XbaI restriction fragment of the c-myc gene, measuring 3.7 kb in length, were rendered double-stranded by DNA polymerase. The HindIII linkers were inserted by T4-DNA-ligase into the resulting blunt-end DNA fragments; after phenol extraction the DNA was treated with the HindIII restriction enzyme. The DNA fragment was subsequently cloned into the pBR322 plasmid at the restriction site of the HindIII restriction enzyme and labelled by nick-translation with ^{125}I-labelled dCTP.

Test capturing probe. The 1.1 kb XbaI-PstI fragment of the c-myc gene was cloned into the M13 mp10 and mp11 phage cloning vectors between the restriction sites of the XbaI and PstI restriction enzymes and affixed to the nitrocellulose filter (150 ng DNA/dia 1 cm).

(b) *Determination of the standard curve*

The sample used for determination of the standard curve consisted of an alkaline-denatured pBR322 clone of the c-myc gene. The sandwich hybridization solution to which the above test probes were added consisted of 4 × SSC, 1 × Denhardt solution, 200 μg/ml herring sperm DNA and 0.2% SDS. Hybridization took place at 65°C for 17–19 hours, whereafter the filters were washed in the wash solution (0.1 × SSC 0.2% SDS) at 50°C. The label attached to the sandwich hybrids was then counted in the gamma counter.

Table 1

Sample molecules/test	*cpm* c-myc-filter
0	40
10^6	75
5×10^6	190
10^7	340
10^8	2200

c) *Determination of the number of genes*

The samples comprised 1) cells from a human placenta and 2) Colo 320 cells, which can be obtained e.g. from the ATCC culture collection (ATCC–CCC220). DNA was isolated from both samples, and the same quantity of cell DNA, denatured by alkaline boiling, was added to both tests. Alkaline denaturation hydrolyzed any RNA present in the sample.

The test was performed by adding to each sample both the c-myc and c-Ki-rasl filters and the two labelled reagents, enabling both the standard and test DNA to be measured for each sample. On the basis of c-Ki-rasl determinations, each test was found to contain the same quantity of DNA and it can be deduced that the c-myc gene in Colo 320 cells is present in about 16–20 higher copy number than in the normal situation. The results are shown in Table 2.

Table 2

Sample	*c-Ki-rasl filter* cpm*	*c-myc filter* cpm*	number
Human placental cells	486	340	10^7
Colo 320 cells	432	3205	1.6×10^8

*the reading obtained from the blank filter has been subtracted from the readings.

EXAMPLE 2

Quantification of amplified gene

a) *Nucleic acid reagents and their preparation*

STANDARD PROBES

Cell standard nucleic acid. The control nucleic acid was taken from the promoter area of the metallothionine gene in the mouse, i.e. the MT gene, and the DNA immediately upstream of it. The structure of the MT gene has been described by Pavlakis and Hamer, PNAS *80*, pp. 397–401, 1983. The reference nucleic acid fragment is available e.g. cloned into the pBPV–MMTneo(432–12) vector (ATCC 37224) and can be obtained, for example, from the ATCC culture collection.

Further treatment of cell standard nucleic acid. The MT gene described above was treated with KpnI, BglII and EcoRI restriction enzymes for subcloning into the pAT153 plasmid. The KpnI tail was converted into a HindIII tail with a linker.

Standard detector probe. The EcoRI-KpnI-(HindIII) fragment measuring about 1.2 kb and located upstream of the promoter area of metallothionine gene was cloned to the pAT153 plasmid between the restriction sites of the EcoRI and HindIII restriction enzymes and labelled by nick-translation with ^{32}P-labelled nucleoside triphosphates.

Standard capturing probe. The 0.8 kb KpnI-BglII fragment comprising the promoter area of the metallothionine gene and the area upstream of it was cloned into the M13 mp18 and M13 mp19 phage vectors between the restriction sites of the KpnI nitrocellulose filter.

TEST PROBES

Test nucleic acid. The probe pair for the sandwich hybridization test was prepared using the commercially available pMTVdhfr plasmid (Bethesda Research Laboratories, product No. 5369SS), the structure of which is described by Lee et al., Nature *294*, pp. 228–232, 1981.

Further treatment of test nucleic acid. The pMTVdhfr plasmid containing cDNA of the dihydrofolate reductase (DHFR) gene was treated with HindIII and BglII restriction enzymes.

Test detector probe. The HindIII-BglII fragment, measuring 0.75 kb and corresponding to the area coding for the DHFR gene of the pMTVdhfr plasmid, was inserted into the plasmid pAT153 vector between the restriction sites of the HindIII and BamHI restriction enzymes and labelled by nick-translation with ^{32}P-labelled nucleoside triphosphates.

Test capturing probe. A HindIII fragment measuring 1.4 kb taken from the MMTV gene area of the pMTVdhfr plasmid was cloned into the M13 mp18 and M13 mp19 phage vectors.

b) *Determination of the standard curve*

The sample used for the test was purified DNA from the pMTVdhfr plasmid. The test itself was carried out as described in Example 1b except that a liquid scintillation counter was used for counting. The resulting standard curve is shown in Table 3.

Table 3

Sample molecules/test	*cpm* DHFR filter
0	17
10^6	45
3×10^6	79
10^7	210

c) *Determination of the number of genes*

Cell lines (derived from the mouse fibroblast cell NIH 3T3 and available from the ATCC culture collection under the number CRL 1658) which has been transfected with different quantities of cDNA corresponding to the mRNA of the DHFR gene were cultured on cell culture plates and used as the sample. The cells were lysed using sodium dodecyl sulphate and their DNA was sheared by squeezing through a fine hypodermic needle from a syringe. A 250 μl sample corresponding to about 10^6 cells was taken from the homogenate and 50 μl NaOH added. The sample was boiled and neutralized with acetic acid and the hybridization mixture. The total volume was 0.5 ml. All the probes described above were added simultaneously and a so-called blank filter was added as a background control. Hybridization, washing and label counting were done as in Example 1b except that a liquid scintillation counter was used for counting. The results are shown in Table 4.

Table 4

Cell	MT	No.of cells	DHFR		
	cpm*	in the sample	cpm*	No. of molecules in the sample	No. of copies
Control cell (No DHFR-cDNA)	182	1.05×10^6	21	$< 10^6$	-
Line I	138	0.9×10^6	80	3×10^6	3
Line II	210	1.25×10^6	732	5×10^7	40

* cpm: the reading given by the blank filter has been subtracted

The MT gene is an internal marker which measures the number of cells present in a sample. The results show that in this test 10^6 cells gave an MT-specific signal of 165+20 cpm. The DHFR reagents measure the quantity of DHFR-cDNA. The number of cells was deduced from the MT-specific signal. It was thus possible to determine the number of DHFR-cDNA copies in different cell lines as shown in Table 4.

EXAMPLE 3

Quantification of messenger RNA

a) *Nucleic acid reagents and their preparation*

Using the test probes described in Example 2 it is also possible to measure the quantity of mRNA derived from DHFR-cDNA. The structure of the pMTVdhfr plasmid is such that transcription of the DHFR gene begins at the MMTV promoter. The resulting messengers are about 1.0 kb in length. Of this, about 0.25 kb are derived from the MMTV promoter area and the rest from DHFR-cDNA (Lee et al., Nature *294*, pp. 228–232, 1981).

STANDARD PROBES

The cell standard nucleic acid, standard detector and standard capturing probe were as described in Example 2.

TEST PROBES

The test nucleic acid, test detector and test capturing probe were as described in Example 2.

b) *Determination of the standard curve*

The sample used for standard curve determination consisted of messenger RNA corresponding to the dihydrofolate reductase gene produced by in vitro transcription. The DNA needed for transcription was prepared by subcloning the 1.4.kb HindIII fragment of the MMTV promoter of the pMTVdhfr plasmid and the 0,75 kb KindIII-BglII fragment (DHFR-cDNA) next to each other into the pSP64 plasmid (Promega Biotec) between the restriction sites of the HindIII and BamHI restriction enzymes. The sample RNA was stored in 0.2% SDS aqueous solution.

The sandwich hybridization test was carried out as described in Examples 1b and 2b but denaturation was omitted.

Table 5

Sample molecules/test	*cpm* DHFR filter
0	20
5×10^6	65
10^7	130
5×10^7	390
10^8	653

c) *Determination of the number of messenger RNA molecules*

The number of messenger RNA molecules corresponding to the DHFR gene was determined from the cell lines described in Example 2.

The cells were lysed using sodium dodecyl sulphate and their DNA was sheared slightly by squeezing through a fine hypodermic needle from a syringe. A 250 µl sample of the homogenate was taken corresponding to about 5×10^6 cells. The homogenate was then added to the sandwich hybridization test without denaturation. Sandwich hybridization took place as described in Examples 2c and 1b, except that only the DHFR probes were added to the hybridization solution. In a parallel sample of 250 µl of homogenate, the cell number was determined using the MT probe as described in Example 2c.

The results are shown in Table 6.

Table 6

Cell	MT	Cell number	DHFR		
	cpm*	in the sample	cpm*	No of molecules in the sample	No. per cell
Line I	380	3.5×10^6	1465	3.45×10^8	100
Line II	430	4.2×10^6	4800	2×10^9	500

*cmp: The reading given by the blank filter has been subtracted.

The results showed that cell line I produced per cell about 100 messenger RNA molecules from the DHFR genes and cell line II produced about 500 messenger RNA molecules from the DHFR genes.

EXAMPLE 4

Quantification of amplified gene by solution hybridization

a) *Nucleic acid reagents and their preparation*

STANDARD PROBES

The cell standard nucleic acid, standard detector and standard capturing probe were as described in Example 2. The 1.2 kb EcoRI-KpnI-(HindIII) fragment in pAT153 was labelled by nick-translation with ^{125}I-labelled deoxycytidine. The 0.8 kb KpnI-BglII fragments in M13 mp18 and M13 mp19 were modified with biotin using the Photoprobe™ reagent (Vector Laboratories, CA, USA, product No SP-1000).

TEST PROBES

The test nucleic acid, test detector and test capturing probe were as described in Example 2. The 0.75 kb HindIII-BglII fragment in pAT153 was labelled with ^{125}I-labelled deoxycytidene. The 1.4 kb HindIII fragments in M13 mp18 and M13 mp19 were biotinylated using Photoprobe™ as above.

b) *Determination of the standard curves*

A cell standard curve was prepared using a known amount of cells, from which the hybridization signal was measured using the standard probes recognizing the MT-gene. A test nucleic acid standard curve was prepared with the pMTVdhfr plasmid and the test probes recognizing this plasmid. Hybridizations were carried out in 200 µl of a solution consisting of 0.6 M NaCl, 20 mM phosphate buffer, pH 7.5, 1 mM EDTA, 4% polyethylene glycol (PEG 6000) for 1.5 hours at 70°C. After the reaction 50 µm of streptavidin-agarose (Betheseda Research Laboratories, Maryland, USA, product No. 5942SA), and 1 M NaCl, 10 mM sodium phosphate, pH 7.5, 1 mM EDTA was added to a final volume of 500 µl. The hybrids were collected on the streptavidin-agarose at 37°C for 15 min. The agarose was washed once for 5 min. with the buffered 1 M NaCl solution at 37°C and twice for 2 min. with 15 mM NaCl, 1.5 mM sodium citrate at 55°C. The amount of bound hybrids was determined by measuring the agarose in a gamma counter. (Syvänen et al., Nucleic Acids Res. *14*, 5037–5048, 1986). The results are shown in table 7 and 8.

Table 7

Sample cells/test	*cpm* MT probes
$0{,}8 \times 10^6$	162
$1{,}6 \times 10^6$	216
3×10^6	298

Table 8

Sample molecules/test	*cpm* DHFR probes
106	148
5×10^6	394
5×10^7	2240

c) *Determination of the number of genes*

Samples of the cell lines described in Example 2 were treated in a similar way, except that the volume per sample corresponding to approximately 2×10^6 cells was 125 µ. The determinations of number of cells and number of test nucleic acid molecules were carried out in separate vials by adding the cell sample, the appropriate detector and capturing probes, and the components of the hybridization mixture to a final volume of 200 µl. Control assays without cell standard or test DNA were included. Hybridization, collection of hybrids, washing and measurement was done as described in Example 4b. The results were read from standard curves prepared in parallel as described in Example 4b. The results are shown in Table 9.

Table 9

Cell	MT		DHFR		
	cpm*	No of cells in the sample	cpm*	No. of molecules in the sample	No. of copies
Control cell	253	2.3×10^6	73	$< 10^5$	-
Line I	210	1.5×10^6	233	3.8×10^6	3
Line II	237	2.1×10^6	3059	8.8×10^7	42

* cpm: values from control assays without cell standard or test nucleic acid have been subtracted.

CLAIMS

1. A quantitative method for determination of nucleic acid molecules by a sandwich or solution hybridization method, characterized in that the number of given nucleic acid molecules per given unit is determined by comparing the number of the test nucleic acid molecules potentially present in several copies in the unit to the number of chosen standard nucleic acid molecules advantageously present in a constant number per same unit.

2. The method according to claim 1 characterized in that the nucleic acids present in the sample

a) are rendered, if necessary, into a form whereby they can participate in the hybridization reaction,

b) any nucleic acids potentially disturbing the hybridization reaction are rendered, if necessary, into a form whereby they cannot interfere with the hybridization test,

c) are brought into contact, either undivided or, when necessary divided, with at least one test probe pair sufficiently homologous to the nucleic acid potentially present in several copies and with at least one chosen and suitable standard probe pair sufficiently homologous to the nucleic acid molecule advantageously present in a constant number; the detector probes of the said test probe pair and said standard probe pair are labelled with a suitable, detectable label and the capturing probes have been affixed to a suitable carrier or a substance has been affixed to the said capturing probes which enables isolation of the resulting hybrids;

d) after the hybridization reaction or reactions have taken place the test hybrid and standard hybrid are separated when necessary and the said attached label measured; the number of nucleic acid molecules per given unit is obtained by comparing the test and standard nucleic acid numbers.

3. The method according to Claims 1 and 2, characterized in that the test and the standard nucleic acids are deoxyribonucleic acids.

4. The method according to claims 1 and 2, characterized in that the test nucleic acid is ribonucleic acid and the standard nucleic acid is deoxyribonucleic acid.

5. The method according to Claims 1 and 2, characterized in that the test and standard nucleic acids are ribonucleic acids.

6. The method according to Claims 1 and 2, characterized in that the test nucleic acid is deoxyribonucleic acid and the standard nucleic acid is ribonucleic acid.

7. The method according to Claims 1, 2, 3 and 4, characterized in that the detector probe of the standard probe pair—a recombinant plasmid comprising a 1.1 kb BglII-BglII fragment of the HindIII fragment of the human c-Ki-rasl gene, said HindIII fragment being cloned into the pBR322 plasmid and said BglII-BglII fragment being subcloned into the restriction site of the BamHI restriction enzyme of the pBR322 plasmid—and the capturing probes—recombinant phages

comprising a 0.5 kb BglII-HindIII fragment of the HindIII fragment of the human c-Ki-rasl gene, said HindIII fragment being cloned into the pBR322 plasmid and said BglII-HindIII fragment being subcloned into the M13 mp10 and M13 mp11 phage vectors between the restriction sites of the BamHI and HindIII restriction enzymes—are brought, either individually or together with the test probe pair, into contact with an undivided or when necessary divided nucleic acid sample.

8. The method according to Claims 1, 2, 3 and 4 characterized in that the detector probe of the standard probe pair—a recombinant plasmid comprising a 1.2 kb EcoRI-KpnI(HindIII) fragment from upstream of the promoter area of the mouse metallothionine gene, which fragment has been subcloned into the pAT153 plasmid between the restriction sites of the EcoRI and HindIII restriction enzymes—and the capturing probes—recombinant phages comprising a 0.8 kb KpnI-BglII fragment from the promoter area of the metallothionine gene and the area upstream of it, which fragment has been subcloned into the M13 mp18 and M13 mp19 phage vectors between the restriction sites of the KpnI and BamHI restriction enzymes—are brought, either individually or together with the test probe pair, into contact with an undivided or when necessary divided nucleic acid sample.

9. The method according to Claims 1, 2, 3, 6, 7 and 8, characterized in that in order to determine the degree of amplification of the c-myc oncogene and/or the number of messenger RNA molecules corresponding to this gene, the detector probe of the test probe pair—a recombinant plasmid comprising a 3.7 kb HindIII-XbaI fragment of the c-myc gene, said fragment being subcloned into the pBR322 plasmid at the restriction site of the HindIII restriction enzyme—and the capturing probes—recombinant phages comprising a 1.1 kb XbaI-PstI fragment of the c-myc gene, which fragment has been subcloned into the M13 mp10 and M13 mp11 vectors between the restriction sites of the XbaI and PstI restriction enzymes—are brought, either individually or together with the standard probe pair, into contact with an undivided or when necessary divided nucleic acid sample.

10. The method according to Claims 1, 2, 3, 6, 7 and 8, characterized in that in order to determine the degree of amplification of the dihydrofolate reductase or DHFR gene and/or the number of messenger RNA molecules corresponding to this gene, the detector probe of the test probe pair—a recombinant plasmid comprising a 0.75 kb HindIII-BglII fragment coding for the DHFR gene of the pMTVdhfr plasmid, which fragment has been subcloned into the pAT153 plasmid vector between the restriction sites of the HindIII and BamHI restriction enzymes—and the capturing probes—recombinant phages comprising a 1.4 kb HindIII fragment of the MMTV gene area of the pMTVdhfr plasmid, which fragment has been subcloned into the M13 mp18 and M13 mp19 phage vectors at the restriction site of the HindIII restriction enzyme—are brought, either individually or together with the standard probe pair, into contact with an undivided or when necessary divided nucleic acid sample.

11. A reagent kit for the quantitative determination of nucleic acid molecules characterized in that the kit contains at least one test probe pair and at least one standard probe pair, the detector probes of both the test probe pair and the standard probe pair being labelled with a suitable label and the capturing probes having been affixed to a suitable carrier or a substance having been affixed to the said capturing probes, enabling isolation of sandwich hybrids.

12. The reagent kit according to Claim 11, characterized in that the detector probe of the test probe pair used for the determination of the degree of amplification of the c-myc oncogene and/or the number of messenger RNA molecules corresponding to this gene is a recombinant plasmid comprising a 3.7 kb HindIII-XbaI restriction fragment of the c-myc gene, which fragment has been subcloned into the pBR322 plasmid at the restriction site of the HindIII restriction enzyme, and the capturing probes are recombinant phages comprising a 1.1 kb XbaI-PstI fragment of the c-myc gene, which fragment has been subcloned into the M13 mp10 and M13 mp11 phage vectors between the restriction sites of the XbaI and PstI restriction enzymes, and the detector probe of the standard probe pair is a 1.1 kb BglII-BglII fragment of the HindIII fragment of the human c-Ki-rasl gene, said HindIII fragment having been cloned into the pBR322 plasmid and said BglII-BglII fragment having been subcloned into the pBR322 plasmid at the restriction site of the BamHI restriction enzyme, and the capturing probes are recombinant phages comprising a 0.5 kb BglII-HindIII fragment of the HindIII fragment of the c-Ki-rasl gene, said HindIII fragment having been subcloned into the pBR322 plasmid of the c-Ki-rasl gene, and said BglII-HindIII fragment having been subcloned into the M13 mp10 and M13 mp11 phage vectors between the restriction sites of the BamHI and HindIII restriction enzymes.

The reagent kit according to Claim 11, characterized in that the detector probe of the test probe pair used for determination of the degree of amplification of the dihydrofolate reductase or DHFR gene and/or the number of messenger RNA molecules corresponding to this gene is a recombinant plasmid comprising a 0.75 kb HindIII-BglII fragment coding for the DHFR gene of the pMTVdhfr plasmid, which fragment has been subcloned into the pAT153 plasmid vector between the restriction sites of the HindIII and BamHI restriction enzymes, and the capturing probes are recombinant phages comprising a 1.4 kb HindIII fragment of the MMTV gene area of the pMTVdhfr plasmid, which fragment has been subcloned into the M13 mp18 and M13 mp19

phage vectors at the restriction site of the HindIII restriction enzyme, and the detector probe of the standard probe pair is a recombinant plasmid comprising a 1.2 kb EcoRI-KpnI fragment from upstream of the promoter area of the mouse metallothionine gene, which fragment has been subcloned into the pAT153 plasmid between the restriction sites of the EcoRI and HindII restriction enzymes, and the capturing probes are recombinant phages comprising a 0.8 kb KpnI-BglII fragment of the metallothionine gene formed by the promoter area and the area upstream of it, which fragment has been subcloned into the M13 mp18 and M13 mp19 phage vectors between the restriction sites of the KpnI and BamHI restriction enzymes.

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